CLINICAL TRACK

*H*ypertrophy Regression with N-*A*cety*L*cys*T*eine in *H*ypertrophic *C*ardio*M*yopathy (HALT-HCM): A Randomized Placebo Controlled Double Blind Pilot Study

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***Running title*:** NAC and Cardiac Hypertrophy and Fibrosis in HCM

Subject Terms:
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ABSTRACT

***Rationale*:** Hypertrophic cardiomyopathy (HCM) is a genetic paradigm of cardiac hypertrophy. Cardiac hypertrophy and interstitial fibrosis are important risk factors for sudden death and morbidity in HCM. Oxidative stress is implicated in the pathogenesis of cardiac hypertrophy and fibrosis. Treatment with anti-oxidant N-acetylcysteine (NAC) reverses cardiac hypertrophy and fibrosis in animal models of HCM.

***Objective*:** To determine effect sizes of NAC on indices of cardiac hypertrophy and fibrosis in patients with established HCM.

***Methods and Results*:** *H*ypertrophy Regression with N-*A*cety*L*cys*T*eine in *H*ypertrophic *C*ardio*M*yopathy (HALT-HCM) is a double blind randomized, sex-matched, placebo-control single center pilot study in patients with HCM. HCM patients, who had a left ventricular wall thickness of ≥15 mm, were randomized either to a placebo or to NAC (1:2 ratio, respectively). NAC was titrated up to 2.4 g per day. Clinical evaluation, blood chemistry, and six-minute walk test were performed every 3 months, and electrocardiography, echocardiography, and cardiac magnetic resonance imaging (CMR), the latter whenever not contraindicated, before and after 12 months of treatment. 85 out of 232 screened patients met the eligibility criteria, 42 agreed to participate; 29 were randomized to NAC and 13 to placebo groups. Demographics, echocardiographic, and CMR phenotypes at the baseline between the two groups were similar. Whole exome sequencing in 38 patients identified a spectrum of 42 pathogenic variants in genes implicated in HCM in 26 participants. Twenty-four patients in the NAC and eleven in the placebo groups completed the study. Six severe adverse events occurred in the NAC group but were considered unrelated to NAC. The effect sizes of NAC on the clinical phenotype, echocardiographic, and CMR indices of cardiac hypertrophy, function, and extent of late gadolinium enhancement, a surrogate for fibrosis, were small.

***Conclusions*:** Treatment with NAC for 12-months had small effect sizes on indices of cardiac hypertrophy or fibrosis. The small sample size of the HALT-HCM study hinders from making firm conclusions about efficacy of NAC in HCM.
Clinical Trial Registration: NCT01537926

Keywords:
Hypertrophic cardiomyopathy, hypertrophy, fibrosis, N-acetylcysteine, sudden cardiac death, cardiac magnetic resonance imaging,

Nonstandard Abbreviations and Acronyms:

CADD	Combined annotation dependent
CMR	Cardiac magnetic resonance imaging
CVA	Cerebrovascular accident
ECV	Extracellular volume fraction
EDV	End diastolic volume
HCM	Hypertrophic cardiomyopathy
ITT	Intention to treat
LGE	Late gadolinium enhancement
LVED	Left ventricular end diastolic
LVMI	Left ventricular mass indexed to body surface area
NAC	N-acetylcysteine
NYHA	New York Heart Association
PP	Per protocol
SCD	Sudden cardiac death
SNV	Single nucleotide variants
WES	Whole exome sequencing

INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a hereditary disease of the myocardium caused mainly by mutations in genes encoding sarcomere proteins. [1] It is an important cause of sudden cardiac death (SCD) in the young and a major cause of morbidity in older individuals, primarily because of cardiac arrhythmias and heart failure with preserved ejection fraction. [1] HCM is clinically diagnosed by the presence of unexplained left ventricular hypertrophy and pathologically by the presence of myocyte disarray and interstitial fibrosis. [1] Cardiac hypertrophy is the quintessential clinical phenotype of human HCM and an important determinant of mortality, morbidity and the risk of SCD. [2-5] Likewise, interstitial fibrosis has been associated with clinical events, including cardiac arrhythmias in HCM. [6-9] Current pharmacological therapies are not effective in preventing, attenuating, or reversing cardiac hypertrophy and fibrosis in HCM.

Genetic and pharmacological interventions in animal models of HCM have established the potential for reversibility of cardiac hypertrophy and fibrosis. [10-15] N-acetylcysteine (NAC) is a precursor to glutathione, which is the most abundant intracellular thiol pool against oxidative stress in the body. Treatment with NAC has been shown to reverse established cardiac hypertrophy and fibrosis in independent studies in animal models of HCM. [15-17] Treatment with NAC also attenuates cardiac hypertrophy induced by pressure overload, administration of angiotensin II, and altered myocardial metabolism. [18-20] The beneficial effects of NAC in models of cardiac hypertrophy and dysfunction are in accord with the important roles of oxidative stress-responsive signaling pathways, myocardial metabolism, and oxidative modification of myofilament proteins in the pathogenesis of cardiac hypertrophy. [17 18-20] Extensive clinical use and safety profile of NAC in humans and the results of the experimental studies in the animal models of HCM, identify NAC as an attractive agent for potentially attenuating and reversing cardiac phenotypes in human patients with HCM. The purpose of this feasibility study, dubbed as "*H*ypertrophy Regression with N-*A*cety*L*cys*T*eine in *H*ypertrophic *C*ardio*M*yopathy (HALT-HCM)" was to determine recruitment, accrual, retention and compliance rates, tolerability, safety, and side effects, as well as the effect sizes of NAC on the indices of cardiac hypertrophy and fibrosis in patients with HCM.

METHODS

Institutional Review Board of the University of Texas Health Science Center approved the study. Each participant signed a consent form. The study was registered at the ClinicalTrials.gov web site (NCT01537926). Anonymized data that support the findings of this study are available from the corresponding author upon reasonable request

Eligibility criteria.
Eligibility criteria are described in Table 1. In brief, adult patients with an established diagnosis of HCM, defined as primary cardiac hypertrophy with a left ventricular end diastolic (LVED) wall thickness of ≥15 mm on a 2-dimensional echocardiogram, non-dilated LV cavity, and preserved LV systolic function, were considered eligible. Those allergic to NAC, a recent history of septal ablation or plan to undergo myectomy in the near future, known phenocopy conditions, and concomitant diseases were excluded. Patients with devices, such as pacemakers and cardioverter/defibrillators (ICDs) and those expected to receive a device during the course of the study were excluded from the CMR part of the study.

TABLE
Eligibility Criteria

Inclusion criteria:

- Established diagnosis of HCM, defined as the presence of primary cardiac hypertrophy with a left ventricular end diastolic (LVED) wall thickness of ≥15 mm, a non-dilated LV cavity, and preserved LV systolic function on a 2D echocardiogram

Exclusion criteria:

- Hypersensitivity to NAC
- Individuals younger than 18 years old
- Known phenocopy conditions
- History of (with the last 6 months) or planned transcatheter (alcohol) septal ablation or surgical myectomy
- Patients who are likely to receive a pacemaker or an ICD implantation during the study period:
  - Patients with advanced atrioventricular block or severe bradycardia
  - Patients with serious ventricular arrhythmias
- Known causal mutations but an LVED wall thickness of <15 mm
- Patients with concomitant diseases
  - Coronary artery disease, defined as >70% luminal diameter stenosis in any of the major coronary arteries (if known)
  - Valvular heart diseases (more than mild aortic stenosis and mitral regurgitation
  - Uncontrolled hypertension, defined as systolic blood pressure of ≥140 mmHg and diastolic blood pressure of ≥90 mmHg on medication (mean values of three measurements at rest)
  - Other significant medical problems, such as moderate to severe chronic renal failure (GFR<45 ml/min/1.73m^2), advanced liver disease, cancer, or other disabling conditions
- Pregnant women, nursing mothers and those who plan pregnancy during the study period
- Patients with active asthma
- Cardiac contra-indications for MRI (for CMR studies only), including patients with devices such as pacemakers and ICDs

Data management.
REDCap (Research Electronic Data Capture) tools, hosted at UTHealth, were used for collecting and managing data. REDCap is a secure, web-based application designed to support data capture for research studies. [21]

Randomization.
The study statistician prepared a block randomization schedule, which balanced the distribution of patients in the study arms 2:1 (NAC group vs. placebo group) and with respect to patient gender. The randomization

schedule was provided to the study pharmacist. The data manager regularly checked to ensure proper utilization of the randomization program.

Dosing of NAC and placebo.

NAC was prepared by a compound pharmacy as 600 mg capsules along with identically appearing placebo capsules. Active and placebo capsules were coated to minimize the distinction between the two capsules based on odor and taste. The compound pharmacy labeled the study drug based on study identification number and randomization. NAC was administered at 600 mg orally every 12 hours for the first 3 months and then was increased to 1,200 mg twice per day for an additional 9 months. Thus, the total duration of treatment with NAC or matching placebo was 12 months.

Compliance.

Compliance with the study drug was determined by counting the number of unused pills in every visit and interviewing the participants.

Genetic analysis.

Exome sequencing was performed using Illumina sequencing platforms and analyzed by the investigators, as described. [22, 23] In brief, the Sequence reads were mapped to the reference Human Genome version 19 (hg19). Single nucleotide variants (SNVs) and small/insertion deletion (indels) were called according to GATK best practices pipeline (https://software.broadinstitute.org/gatk/best-practices/). Functional annotation of the variant list for each exome was performed utilizing ANNOVAR software (http://annovar.openbioinformatics.org/). Rare variants in 19 genes (*ACTC1, ACTN2, CALR3, CSRP3, JPH2, MYBPC3, MYH7, MYL2, MYL3, MYOZ2, NEXN, PLN, PRKAG2, TCAP, TNNI3, TNNT2, TPM1, TTN, VCL*), implicated in familial HCM (https://ghr.nlm.nih.gov/condition/familial-hypertrophic-cardiomyopathy#genes), were manually curated. Variants meeting the following criteria were considered pathogenic.

1) Minor allele frequency ≤ 0.001, as reported in the Genome Aggregation Database (http://gnomad.broadinstitute.org/);

2) Combined annotation dependent (CADD) phred score, as a measurement of relative pathogenicity, of ≥10 [24]; and

3) A minimum of 10 reads covering the candidate variant and the genotype quality of 99 (a Phred-scaled confidence that the genotype assignment is correct. It is equal to 1% error rate).

Clinical evaluation.

A medical history was obtained, New York Heart Association functional class was recorded, and physical examination was performed in every participant. The participants also completed a 6-minute walk test. A blood chemistry panel comprised of electrolytes, BUN, creatinine, liver enzymes and lipids were obtained at the baseline and every 3 months until the completion of the study. Twelve-lead electrocardiograms were obtained in all participants at baseline and completion of the study.

Echocardiographic imaging and analysis.

The patients were imaged in the left lateral decubitus position with an ultrasound system equipped with a multifrequency transducer. A complete echocardiographic study was performed per standard views. American Society of Echocardiography/European Association of Cardiovascular Imaging (ASE/EACVI) guidelines were applied to measure biplane LV volumes, mass, and ejection fraction (EF) as well as left atrial maximum volume. [25] From the apical window, pulsed-wave Doppler was used to record mitral inflow for three to five cardiac cycles at the level of the mitral valve annulus and tips. [26] Pulmonary venous flow was recorded using color Doppler guidance. Tissue Doppler (TD) was applied to record mitral annular velocities at the septal and lateral sides of the annulus. The resulting annular velocities by pulsed-wave Doppler were recorded for five cardiac cycles at a sweep speed of 100 mm/s. Tricuspid regurgitation velocity was recorded by continuous wave (CW) Doppler from multiple windows and the peak velocity

was noted. Inferior vena caval diameter and its collapse as well as hepatic venous flow were recorded from the subcostal views. [27] Echocardiographic measurements were performed offline (Digisonics EC, Houston, TX) without knowledge of randomization groups, clinical and CMR findings.

Mitral inflow from tips level was analyzed for peak early (E), and late (A) diastolic velocities, E/A ratio, and deceleration time of mitral E velocity. Mitral A duration was measured at the level of mitral annulus. [26] Pulmonary venous flow was analyzed for peak systolic velocity, peak diastolic velocity, peak amplitude and duration of atrial reversal velocity (Ar) and the difference between Ar velocity duration and mitral A duration or Ar-A duration. [26] Mitral annulus early (e') and late (a') diastolic velocities were measured at septal and lateral mitral annulus, and E/e' ratios were computed. Measurements were averaged over three cardiac cycles.

CMR.

1.5 T whole body imagers were utilized in all cardiac MRI studies. LV cine imaging was performed using steady-state free precession sequences in long and short axes with temporal resolution less than or equal to 40 msec. LV structural and functional measures included LV mass, end diastolic volume (EDV), end systolic volume (ESV), EF and stroke volume based on contours of the endocardial and epicardial contours of the left ventricle using CMR software (Circle Cardiovascular Imaging, Calgary, Canada). T1 mapping of the myocardium before and after 0.15 mmol/kg gadolinium contrast administration was performed. Details of the T1 mapping method and cardiac MRI methods using methods that were described previously. [28] Briefly, T1 mapping indices including pre (native) and post-contrast T1 times at 12 and 25 minutes, partition coefficient (λ), and extracellular volume fraction [ECV=100×λ×(1-hematocrit)] were assessed using a single-breath hold modified Look-Locker inversion recovery (MOLLI) sequence. [29] Late gadolinium enhancement (LGE) images were obtained at the same slice locations as the cine images in the short and long axis imaging planes using fast gradient echo imaging beginning 15 minutes after the administration of the gadolinium contrast agent. Myocardial scar was defined as focal LGE either in two adjacent short axis slices or in one short axis and a long axis image at a corresponding location. Focal LGE was traced using QMass (version 7.2; Medis, Leiden, the Netherlands) to derive myocardial scar mass.

Endpoints.

The study was a feasibility study, designed primarily to assess the recruitment, retention, and compliance rates, the side effects, and the effect sizes of NAC on echocardiographic and CMR indices of cardiac hypertrophy and fibrosis. Therefore, changes in left ventricular mass index (LVMI), ventricular wall thickness, left ventricular ejection fraction on echocardiography and CMR as well as macroscopic interstitial myocardial fibrosis, as defined by delayed enhancement on CMR following gadolinium contrast administration, were included as secondary end points.

Statistical analysis.

Baseline characteristics of patients randomized to the two study groups were compared. Intention-to-treat (ITT) analysis was applied to compare the clinical, echocardiographic and CMR outcome variables. This was complemented with the per-protocol (PP) analysis to calculate the effect sizes of NAC. For continuous variables, normality of the distribution was tested by Shapiro-Wilk test. For continuous variable with normal distribution groups were compared using two independent sample t test. Continuous variables with non-normal distribution were analyzed with the Kruskal-Wallis test and categorical variables were analyzed by the Chi Square test. Changes in outcome variables between the two time-points were analyzed using longitudinal analyses (Mixed Models). In these models, interaction analysis was performed to determine if the treatment effect varied by phenotype. The effect size of NAC on the echocardiographic and CMR indices were calculated as $(\Delta^{Placebo} - \Delta^{NAC})/SD^{Placebo}$, per Glass's Δ method. [30] SAS 9.4 statistical package (SAS Institute Inc. USA) was used for data analyses.

RESULTS

Recruitment, retention, and compliance.

A total of 232 patients with HCM were screened over a period of 3 years of whom 85 met the eligibility criteria and 147 did not (Figure 1). The main reason for exclusion was a LV wall thickness of < 15 mm (55 patients) on the screening echocardiogram (Figure 1). Twenty-eight of the 85 eligible individuals declined to participate and 15 did not respond to the follow-up enrollment invitations. Forty-two participants (32 males and 10 females) signed inform consents; 29 were randomized to NAC and 13 to placebo groups. The baseline demographics of the study population in each group are shown in Table 2. There were no significant differences in ages, sexes, ethnic backgrounds, body mass indices, mean systolic and diastolic blood pressures and NYHA functional classes or other clinical characteristics between the two groups at enrollment with the exception of a history of smoking, which was higher in the NAC group.

TABLE 2

Baseline Characteristics of the Study Population

	Placebo (±SD)	**NAC (±SD)**	***p* Value**
N	13	29	
Male/Female	10/3	22/7	0.94
Age (years) (mean)	47.6 (15.1)	50.7 (15.0)	0.56
Height (cm) (mean)	173.1 (11.2)	174.3 (11.4)	0.97
Weight (Kg) (mean)	89.3 (22.9)	92.6 (19.5)	0.71
BSA (m^2) (mean)	2.07 (0.32)	2.12 (0.27)	0.62
BMI (Kg/m^2) (mean)	29.5 (5.4)	30.4 (5.4)	0.71
Ethnic: N (%)			
Hispanic	1 (7.7)	4 (13.8)	0.57
Non-Hispanic	12 (92.3)	25 (86.2)	
Race: N (%)			
Caucasian	10 (76.9)	23 (79.4)	
African American	1 (7.7)	4 (13.8)	0.47
Asian or Pacific Islander	2 (15.4)	1 (3.4)	
Mixed	0 (0.0)	1 (3.4)	
NYHA Functional class: N (%)			
Class I	5 (38.5)	16 (55.2)	
Class II	5 (38.5)	11 (37.9)	0.29
Class III	3 (23.0)	2 (6.9)	
Class IV	0 (0.0)	0 (0.0)	
Past Medical History (history of)			
Cardiac arrest: N (%)	0 (0)	1 (3.4)	0.50
Atrial fibrillation: N (%)	2 (15.4)	3 (10.3)	0.64
Other cardiac arrhythmias: N (%)	8 (61.5)	19 (65.5)	0.80
Syncope: N (%)	4 (30.1)	8 (27.6)	0.83
ICD implantation: N (%)	3 (23.1)	11 (37.9)	0.34
Diabetes mellitus: N (%)	0	3 (10.3)	0.23
Hypertension: N (%)	5 (38.5)	12 (41.4)	0.86
Dyslipidemia: N (%)	5 (38.5)	13 (44.8)	0.70

Ex-smoker & Current smoking N (%)	1 (7.7)	13 (44.8)	0.02
History & Physical Examination			
Heart rate (bpm) (mean)	67.2 (11.7)	62.4 (11.2)	0.14
Systolic blood pressure (mmHg) (mean)	121.2 (14.9)	126.0 (11.7)	0.30
Diastolic blood pressure (mmHg) (mean)	76.2 (10.8)	79.8 (8.3)	0.41
Chest pain/tightness: N (%)	5 (17.2)	6 (20.7)	0.23
Palpitations: N (%)	2 (15.4)	4 (13.8)	0.89
Systolic ejection murmur: N (%)	5 (38.5)	11 (37.9)	0.97
Pan-systolic murmur: N (%)	1 (7.7)	4 (13.8)	0.57
Laboratory			
Total Cholesterol (mg/dL) (mean)	193.5 (54.5)	169.8 (36.5)	0.18
Triglycerides (mg/dL) (mean)	128.6 (55.7)	170.9 (150.4)	0.87
Medication			
Beta-blockers: N (%)	9 (69.2)	20 (69.0)	0.99
Statins: N (%)	3 (23.1)	8 (27.6)	0.76
Anti-arrhythmic drugs: N (%)	1 (7.7)	3 (10.3)	0.79
RAAS drugs (%)	2 (15.4)	9 (31.0)	0.29

Data are presented as mean ±SD, unless specified
For comparison of categorical data *p* value is based on Chi-Square test or Fisher's exact test.
For comparison of continuous data *p* value is based on Wilcoxon Rank Sum test
Abbreviations: BSA: Body surface area; BMI: Body mass index; NSVT: Non-sustained ventricular tachycardia; VT: Ventricular tachycardia; ICD: internal cardioverter and defibrillator; RAAS: Renin-angiotensin-aldosterone system

Twenty four out of 29 (83%) patients in the NAC and 11/13 (85%) in the placebo groups completed the 12-month duration of the study. The reasons for drop out were: lost to follow up (4 patients), unable to commit time (one patient), non-study related surgical procedure requiring prolonged hospitalization (one patient), and skin rash (one patient). Demographics of patients who completed the study did not differ between the two groups. Compliance rate, determined by pill counting and patient interview in each visit, was high, as the participants consumed 92±8% (range 66 to 100%) of the prescribed study drug.

Biochemical laboratory values, including blood chemistry, kidney function and liver function tests did not significantly change in either of the study groups during the course of 12 months (data not shown).

Pathogenic variants in the known HCM genes.

Thirty-eight of the 42 participants in HALT-HCM study agreed to genetic testing by whole exome sequencing (WES). Variant analysis led to identification of 42, including 11 novel (not reported in gnomAD database) pathogenic variants in 26 (68%) participants in 19 known genes considered to be causes of HCM (Table 3). *TTN* gene had the highest number of pathogenic variants (23, including 5 novel variants), likely reflective of it enormous size, and all were missense variants with a CADD score of >10. Eight pathogenic variants, including two indels, leading to frameshift; and one splice junction variant, in the *MYBPC3* gene were identified in 9 participants. Eleven participants had two or more pathogenic variants in the known HCM genes, including one individual, who had 4 pathogenic, including 3 novel variants in the *TTN*, and one pathogenic variant in the *MYBPC3* genes.

TABLE 3

Pathogenic Variants in the Participants in Known HCM Genes

Gene	Variants with reads ≥10, Genotype quality = 99	Number of individuals	Gnomad AF	CADD Phred Score	ClinVar Clinical Significance
ACTC1	NM_005159:exon3:c.C299T: p.Pro100Leu	1	0.000004	23.2	Uncertain Significance
ACTN2	NM_001103:exon16:c.G1864A: p.Asp622Asn	1	0.0003	35	Conflicting interpretations of pathogenicity
MYBPC3	NM_000256:exon23:c.2373dupG: p.Trp792ValfsTer41	1	0.000018	NA	W792Ter,W792R, Pathogenic/ Likely pathogenic
MYBPC3	NM_000256:exon16:c.G1468A: p.Gly490Arg	1	0.0002	25.1	Conflicting interpretations of pathogenicity
MYBPC3	NM_000256:exon6:c.G655C: p.Val219Leu	1	Not listed	16.74	Pathogenic/ Likely pathogenic
MYBPC3	NM_000256:exon12:c.G1000A: p.Glu334Lys	1	0.0002	35	Conflicting interpretations of pathogenicity
MYBPC3	NM_000256:exon20:c.1928-2A>G	2*	Not listed	16.32	Pathogenic
MYBPC3	NM_000256:exon12:c.1028delC: p.Thr343fs	1	Not listed	NA	Pathogenic
MYBPC3	NM_000256:exon22:c.G2308A: p.Asp770Asn	1	0.000016	35	Pathogenic/ Likely pathogenic
MYBPC3	NM_000256:exon16:c.G1484A: p.Arg495Gln	1	0.00002	27.6	Pathogenic/ Likely pathogenic
MYH7	NM_000257:exon23:c.C2722G: p.Leu908Val	1	0.00003	18.46	Conflicting interpretations of pathogenicity
MYH7	NM_000257:exon21:c.G2330C: p.Arg777Thr	1	Not listed	24.9	Pathogenic
MYL2	NM_000432:exon2:c.G37A: p.Ala13Thr	3*	0.0003	16.11	Conflicting interpretations of pathogenicity
TNNT2	NM_000364:exon10:c.C304T: p.Arg102Trp	3*	0.000004	20.9	Pathogenic/ Likely pathogenic

TTN	NM_001267550:exon326:c.G78394A:p.Asp26132Asn	1	0.00001225	15.71	
TTN	NM_001267550:exon298:c.G58491A:p.Met19497Ile	1	Not listed	13.82	
TTN	NM_001267550:exon117:c.C31384A:p.Pro10462Thr	1	0.000001187	17.89	
TTN	NM_001267550:exon63:c.C18389G:p.Thr6130Arg	1	Not listed	12.97	
TTN	NM_001267550:exon300:c.C59315T:p.Pro19772Leu	1	Not listed	10.15	
TTN	NM_001267550:exon133:c.G32743C:p.Ala10915Pro	1	0.0009620	12.38	
TTN	NM_001267550:exon269:c.C50758G:p.Pro16920Ala	1	0.0002400	14	
TTN	NM_001267550:exon358:c.A106154C:p.Lys35385Thr	1	0.000000 8134	11.53	
TTN	NM_001267550:exon326:c.A74596G:p.Thr24866Ala	1	0.0002035	12.49	
TTN	NM_001267550:exon152:c.C34778T:p.Pro11593Leu	1	0.000008603	18.58	
TTN	NM_001267550:exon335:c.T90913C:p.Tyr30305His	1	0.0001468	16.06	
TTN	NM_001267550:exon358:c.C101281T:p.Arg33761Trp	1	0.00009394	16.42	
TTN	NM_001267550:exon345:c.G95743A:p.Ala31915Trp	1	Not listed	12.5	
TTN	NM_001267550:exon326:c.C85471T:p.Arg28491Cys	1	0.00005074	13.89	Uncertain Significance
TTN	NM_001267550:exon318:c.G67318A:p.Gly22440Ser	1	Not listed	11.25	
TTN	NM_133379:exon46:c.T11289A:p.Asp3763Glu	1	Not listed	16	
TTN	NM_001267550:exon318:c.A67104C:	3*	0.000049	10.46	

	p.Lys22368Asn				
TTN	NM_001267550:exon326:c.G82810A:p.Gly27604Ser	1	0.0004783	23.8	p.Gly27604Arg: Uncertain Significance
TTN	NM_001267550:exon28:c.C5198T:p.Thr1733Met	1	0.0002640	11.69	Uncertain Significance
TTN	NM_001267550:exon358:c.A103591G:p.Lys34531Glu	1	0.00007314	13.21	
TTN	NM_001267550:exon324:c.G68864C:p.Gly22955Ala	1	0.0002547	12.56	

* Family members

Abbreviations: *ACTC1*: Cardiac α actin; *ACTN2*: α Actinin 2; *MYBPC3*: Myosin binding protein C3; *MYH7*: Myosin heavy chain Y; *MYL2*: Myosin light chain 2; *TNNT2*: Cardiac troponin T; *TTN*: Titin; AF: allele frequency; CADD: Combined Annotation Dependent Depletion

Clinical symptoms.

Ten out of 13 and 27/29 participants in the placebo and NAC groups, respectively, were in NYHA functional class I or II (Online Table I). Only 5 patients in the study population were in class III and none in class IV (Online Table I). Distribution of patients among NYHA functional classes did not differ significantly between the placebo and NAC groups at the baseline and during the follow up intervals (Online Table I). The change in NYHA functional class status of each patient during each follow up interval is illustrated in Online Figure I. Frequency or severity of chest pain and palpitations did not change during the study period (data not shown). No patient experienced syncope or near syncope during the study period. All participants completed a 6-minute walk test without experiencing notable symptoms. There were no differences in the mean distance walked at the baseline and the follow up time points between the two groups (Online Figure II).

Electrocardiographic indices.

There were no significant differences in the electrocardiographic findings, including indices of cardiac hypertrophy at the baseline or upon completion of the study (Online Table II). [31]

Echocardiographic phenotypes.

Indices of left ventricular wall thickness, size, and function were not different between the placebo and NAC groups at baseline and upon completion of the study (Table 4 and Online Table III). Echocardiographic data in those who had baseline and 12-months studies were used to calculate the effect sizes. The effect sizes of NAC on indices of cardiac hypertrophy and function, including Doppler indices of left ventricular function were negligible to modest (Table 4). Likewise, when all patients who participated in the study were included in the analysis (intention to treat analysis), there were no discernible differences in echocardiographic or Doppler indices of left ventricular size and function between the placebo and NAC groups in a model that included interaction between the study groups and the follow up time (Online Table III).

TABLE 4

Echocardiographic Phenotypes (Per-Protocol analysis)

	Placebo			NAC			p	$\Delta^{Placebo}-\Delta^{NAC*}$ (95% CI)	SE	Effect size
N	11			24						
M/F	9/2			18/6						
Age (year)	49.35 (15.21)			50.18 (15.86)						
	Baseline	**Follow-up**	**Delta (Δ)**	**Baseline**	**Follow-up**	**Delta (Δ)**				
Body weight (Kg)	88.03 (23.30)	89.44 (22.70)	1.40	90.82 (16.91)	92.94 (17.96)	2.12	0.74	1.58 (-2.82, 4.25)	2.16	0.11
BSA (m2)	2.05 (0.32)	2.07 (0.32)	0.02	2.1 (0.23)	2.12 (0.25)	0.02	0.93	0.00 (-0.04, 0.05)	0.03	0.07
IVST (mm)	18.00 (3.97)	17.82 (4.87)	-0.18	18.88 (4.59)	17.92 (3.3)	-0.96	0.57	0.78 (-0.78, -3.53)	1.35	0.23
LVPWT (mm)	13.55 (3.59)	13.27 (3.8)	-0.27	12.29 (2.69)	13.79 (1.96)	1.50	0.09	1.77 (-0.31, 3.85)	1.02	0.53
Maximum wall thickness (mm)	20.55 (3.24)	20.55 (4.11)	0.00	20.54 (4.42)	19.75 (2.92)	-0.79	0.47	0.79 (-2.97, 1.39)	1.07	0.30
LVEDD (mm)	39.36 (6.42)	39.91 (2.7)	0.55	39.38 (7.41)	39.83 (6.34)	0.46	0.97	0.09 (-5.37, 5.20)	2.60	0.01
LVESD (mm)	22.64 (5.16)	21.55 (3.8)	-1.09	22.13 (6.06)	21.88 (5.29)	-0.25	0.72	0.84 (-3.89, 5.57)	2.32	0.14
EF (%)-mean	69.45 (6.53)	68.55 (5.17)	-0.90	69.23 (6.80)	69.44 (6.12)	0.22	0.59	1.12 (-3.00, 5.24)	2.02	0.20
LA Volume (ml)	74.62 (27.34)	83.58 (27.83)	9.45	89.50 (26.7)	86.08 (31.63)	0.89	0.33	8.56 (-28.63, 11.52)	9.72	0.49
LVM (g)	292.80 (107.50)	290.44 (99.81)	-2.36	269.65 (94.17)	281.98 (81.75)	12.33	0.56	14.69 (-36.32, 65.69)	25.10	0.23
LVMI (g/m2)	141.95 (50.14)	140.41 (47.27)	-1.55	128.48 (43.60)	132.92 (35.41)	4.45	0.63	5.99 (-19.10, 31.10)	12.33	0.19

E (cm/s)	79.04 (18.16)	77.55 (14.24)	4.19	70.53 (20.23)	74.79 (22.4)	3.81	0.85	0.38 (-10.95, 10.19)	5.16	0.02
A (cm/s)	71.00 (25.50)	64.29 (31.31)	1.58	63.04 (26.07)	61.36 (24.09)	-2.09	0.86	0.367 (-19.61, 12.77)	7.77	0.20
E/A	1.28 (0.67)	1.40 (0.54)	0.09	1.27 (0.46)	1.38 (0.56)	0.13	0.94	0.04 (-0.67, 0.75)	0.24	0.05
IVRT (msec)	85.64 (12.85)	86 (18.77)	-2.00	82.25 (13.87)	87.79 (20.86)	2.14	0.59	4.14 (-16.19, 24.48)	9.78	0.16
Septal Sa (cm/s)	5.49 (1.89)	4.69 (1.79)	-1.38	5.16 (0.83)	4.26 (1.63)	-0.88	0.63	0.50 (-1.27, 2.27)	0.83	0.39
Septal Ea (cm/s)	4.49 (2.05)	5.17 (1.48)	-0.02	4.79 (1.42)	4.45 (1.61)	-0.04	0.48	0.02 (-1.68, 1.64)	0.77	0.03
Lateral Sa (cm/s)	5.13 (2.39)	4.53 (1.69)	-0.28	5.53 (1.51)	5.05 (1.84)	0.08	0.74	0.36 (-1.33, 2.05)	0.78	0.23
Lateral Ea (cm/s)	6.17 (2.10)	9.14 (3.27)	2.36	7.00 (2.16)	6.36 (2.35)	0.24	0.02	2.12 (-5.44, 1.21)	1.53	0.64
E/e' ratio	18.80 (9.14)	15.63 (6.33)	-0.47	15.26 (4.63)	18.97 (9.59)	1.88	0.21	2.35 (-5.40, 10.10)	3.69	0.53
LVOT Gradient at rest (mmHg)	10.45 (23.44)	15.73 (28.44)	5.27	14.81 (25.14)	15.75 (25.02)	1.62	0.65	3.65 (-22.05, 14.75)	8.98	0.14
SAM: N (%)	1 (9.09)	3 (27.27)	2	3 (12.50)	5 (20.83)	2	NA	NA	NA	NA

All data are presented as mean (SD), unless specified

* Absolute value of $\Delta^{Placebo}$-Δ^{NAC}

Abbreviations: NAC: N-acetylcysteine; M/F: Male/females; CI: Confidence interval; SE: Standard Error; BSA: Body surface area; IVST: Interventricular septal thickness; LVPWT: Left ventricular posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVESD: Left ventricular end systolic diameter; LVEF: Left ventricular ejection fraction; LA: left atrium; LVM: Left ventricular mass; LVMI: Left ventricular mass indexed to body surface area; E: Mitral valve early inflow velocities; A: Mitral valve inflow late velocities; IVRT: Isovolumic relaxation time; Septal Sa: Systolic mitral annulus velocity measured at septal side of the mitral annulus; Septal Ea: Early diastolic mitral annulus velocity measured at septal side of the mitral annulus; Lateral Sa: Systolic mitral annulus velocity measured at lateral side of the mitral annulus; Lateral Ea: Early diastolic mitral annulus velocity measured at lateral side of the mitral annulus; LVOT: left ventricular outflow tract gradient; SAM: Systolic anterior motion of the mitral leaflet; CI: Confidence interval; SE: Standard error.

CMR.

Data were available in 9 and 15 patients at the baseline in the placebo and NAC groups, respectively, and in 8 and 10 patients at both time points. Data in those who had the baseline and follow up data were used to calculate the effect sizes of NAC on indices of cardiac hypertrophy, fibrosis, and fibrosis (LGE). As shown in Table 5, no large effect sizes on cardiac mass, LVEF, left ventricular mid wall strain, and fibrosis were detected. Likewise, indices of left ventricular wall thickness, cavity size, and function as well as mass were not significantly different between the two groups at baseline and upon completion of the study in the intention to treat analysis (Online Table IV).

TABLE 5

Cardiovascular Magnetic Resonance (CMR) Phenotypes (Per-Protocol analysis)

	Placebo			**NAC**			**p**	**$\Delta^{Placebo}$-Δ^{NAC}* (95% CI)**	**SE**	**Effect size**
N	8			10						
M/F	7/1			7/3						
Age (year)	50.1 (17.9)			48.9 (16.2)						
	Baseline	**Follow-up**	**Delta (Δ)**	**Baseline**	**Follow-up**	**Delta (Δ)**				
MWT (mm)	16.95 (5.66)	17.58 (4.01)	0.63	16.80 (4.77)	17.75 (6.05)	0.95	0.81	0.33 (-2.55, 3.20)	1.36	0.12
LVEDV (ml)	181.42 (43.85)	175.13 (47.92)	-6.29	180.9 (33.31)	179.0 (41.31)	-1.90	0.53	4.39 (-10.29, 19.07)	6.92	0.32
LVESV (ml)	78.20 (27.82)	79.25 (30.89)	1.06	75.7 (18.61)	77.2 (24.29)	1.50	0.93	0.45 (-9.78, 10.67)	4.82	0.04
LVEF (%)	57.48 (9.00)	55.00 (10.95)	-2.48	58.1 (7.11)	57 (8.03)	-1.10	0.50	1.38 (-2.81, 5.56)	1.98	0.27
Mean LV mid wall strain (%)	-13.86 (3.73)	-15.86 (3.75)	-2.16	-15.22 (2.66)	-14.78 (2.37)	0.44	0.05	0.74 (-1.09, 6.30)	1.25	0.08
Myocardial Mass (g)	207.83 (82.38)	221.29 (87.65)	-1.67	214.89 (97.68)	238.22 (99.72)	41.25	0.48	42.92 (-44.51, 130.30)	43.32	2.83
EM (g)	53.67 (45.03)	51.43 (25.53)	-5.17	29.67 (16.86)	38.00 (18.57)	7.00	0.32	12.17 (-13.98, 38.31)	12.00	0.46
EV (%)	22.50 (15.14)	22.29 (5.88)	-0.17	13.89 (5.25)	16.67 (7.84)	1.50	0.64	1.67 (-9.58, 12.92)	5.16	0.14

All data are presented as Mean (SD), unless specified. p values are per mixed model analysis

***** Absolute value of $\Delta^{Placebo}$-Δ^{NAC}

Abbreviations: NAC: N-acetylcysteine; M/F: Male/females; MWT: Maximum wall thickness; LVEDV: Left ventricular end diastolic volume; LVESV: Left ventricular end systolic volume; LVEF: Left ventricular ejection fraction; LA: left atrium; EM: Enhanced myocardium; EV: Percent of myocardium that has scar (percentage EM/MM); CI: Confidence interval; SE: Standard error

Adverse and serious adverse effects.

There was no adverse event. A total of 6 serious adverse events in 5 individuals were recorded in the study population and all occurred in the NAC group (Fisher exact test p=0.155). Two patients developed pneumonia requiring treatment with antibiotics, two patients developed a cerebrovascular accident (CVA), including one who had prior episodes of CVAs and subsequently developed seizure. One patient developed chest pain, which was determined not to be of cardiac origin. None of the serious adverse events was judged to be the known side effect of the study drug.

DISCUSSION

The results of HALT-HCM, a single center randomized placebo-controlled feasibility study, were notable for a relatively slow accrual rate of HCM patients but a high retention and compliance rates upon enrollment. About one fifth of the 232 patients (42 patients) who were screened were recruited and randomized. The compliance and retention rates at 12-month were high, which are notable considering that most patients were asymptomatic or mildly symptomatic and did not have strong incentive to take the study medication and undergo testing. The recruitment, retention, and compliance rates in a single center study of HCM, which is an uncommon disease, might reflect the referral basis of the study population. The findings also document the effect sizes of NAC on echocardiographic and CMR indices of cardiac hypertrophy and fibrosis, which were relatively modest. The study sample size did not provide sufficient power to determine efficacy of NAC in treatment of patients with HCM, except for detecting large changes in the indices of cardiac hypertrophy and fibrosis. Overall, the findings have implications for the design of future efficacy studies in patients with HCM.

The small sample size of the study prohibits from making firm conclusions about efficacy of NAC in HCM. Nevertheless, the effect sizes of NAC, administered for one year at a high dose, on the echocardiographic and CMR indices of cardiac hypertrophy and fibrosis were relatively small. The findings were concordant, regardless of whether the data were analyzed by the ITT or PP approaches. Because the study was performed in patients with established cardiac hypertrophy and fibrosis, the findings might not pertain to evolving cardiac hypertrophy and fibrosis or prevention of the phenotype in HCM. Modest statistical differences in a few echocardiographic indices, including tissue Doppler indices of left ventricular function were noted between the baseline and follow up data in the placebo and treatment groups. However, these differences did not follow a concordant pattern with the related phenotypes and were not consistent across imaging modalities to suggest a clinical significance. Moreover, no changes in the clinical symptoms and exercise tolerance in a 6-min walk test were observed, albeit most patients were asymptomatic or minimally symptomatic and did not have exercise intolerance. There were a higher number of adverse events in the NAC group, but the adverse events did not seem to be related to administration of NAC.

HALT-HCM was designed as a pilot feasibility study and not an efficacy study, It was designed to provide information on recruitment, retention and compliance rates as well as tolerability and the effect sizes of NAC on indices of cardiac hypertrophy and fibrosis for designing future efficacy studies. The plan was to recruit 25 patients to the placebo and 50 patients to the NAC groups. However, only 42 patients participated in one-year long HALT-HCM study. Approximately, half of the eligible patients, who were mostly asymptomatic or mildly symptomatic, declined to participate (Figure 1). Data collected from those who completed the one-year study were used to determine estimates and 95% confidence intervals for the effect size of NAC on echocardiographic and CMR indices of cardiac hypertrophy, function, and fibrosis. These effect sizes were small (Tables 4 and 5). A post-hoc analysis showed that the sample size of the study provided 66% to detect 20% (26 g/m^2) change and 84% power to detect a 25% (33 mg/m^2) change in

the mean LVMI, when α is set at 0.05. The study did not have sufficient power to detect smaller changes, which might be more realistic, given the slow development of cardiac hypertrophy in HCM. Overall, the small sample size of the study on indices of cardiac hypertrophy and fibrosis in HCM suggest that designing and implementation of large-scale efficacy studies with NAC would be challenging.

HCM is a genetically and clinically heterogeneous disease. The spectrum of putative causal genes and mutations in the study participants were in accord with the known genetic heterogeneity of HCM. [1] The abundance of the pathogenic variants in *TTN* was notable but likely reflects the enormous size of its main transcript, coding for ~ 35,000 amino acids. Likewise, several individuals had multiple pathogenic variants in genes known to cause HCM, which is in keeping with the oligogenic basis of a subset of HCM patients. [23] The variants were novel or rare (<1:1000 population frequency in the gnomeAD database) and were considered pathogenic. However, given the study participants were either sporadic cases or members of small families, causality of these variants in HCM could not be ascertained unambiguously. This is particularly the case for the pathogenic variants in the *TTN* gene, because of the abundance of the non-synonymous and truncating *TTN* variants in the general population. [32] Consequently, the pathogenic variants in the *TTN* gene identified in the HALT-HCM population may not be the true causes of HCM in this population and/or might simply contribute or modify expression of the phenotype. [33, 34] Nevertheless, a subgroup analysis in those carrying pathogenic variants in the *TTN* gene also showed no discernible effect of NAC on cardiac phenotype in HCM (Online Table V). Give the small number of the participants with each pathogenic variant and other genes, no additional gene-dependent analysis was performed.

The null findings of the study with regards to the effects of NAC on cardiac hypertrophy and fibrosis are in discord with the results of experimental studies in animal models of HCM, where treatment with NAC was highly effective in attenuating established hypertrophy and fibrosis. [15-17] Such conflicting findings also have been observed in studies of other pharmacological interventions in HCM, including studies with statins and inhibitors of the renin-angiotensin-aldosterone system, where the efficacy of these pharmacological agents in treatment of human patients with HCM has not been substantiated, despite the beneficial effects in animal models of HCM. [10, 11, 13, 35] The differences in the results of experimental therapies in model organisms and clinical trials in humans are likely multifarious and not uncommon. [36-38] The differences in part may reflect extreme genetic heterogeneity of human patients with HCM, as opposed to the model organisms, where a single genetic mutation is introduced in a congenic background to induce the phenotype. Likewise, numerous other biological differences between humans and model organisms, including the confounding effects of the environmental factors and microbiome. [37] Finally, shortcomings of the study design in some of the experimental studies, such as introduction of unconscious biases in group assignment, data acquisition, and interpretation might contribute to failure to extend the findings in the model organisms to humans. [36, 39]

In conclusions, the results of the HALT-HCM, a pilot feasibility study, showed small effect sizes of treatment with a high dose of NAC for 12 months on indices of cardiac hypertrophy and fibrosis in patients with established HCM. A higher number of adverse events occurred in the NAC group, but these events did not seem to be related to administration of NAC. The small sample size of the study did not enable assessing efficacy of NAC. Data on recruitment, retention, compliance and the effect sizes of NAC on indices of cardiac hypertrophy and fibrosis might provide guidance in designing efficacy studies in HCM.

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DISCLOSURE
None

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FIGURE LEGENDS

Figure 1. Consort chart showing screening, recruitment, and randomization process

* Some individuals had more than one exclusion criteria.

NOVELTY AND SIGNIFICANCE

What Is Known?

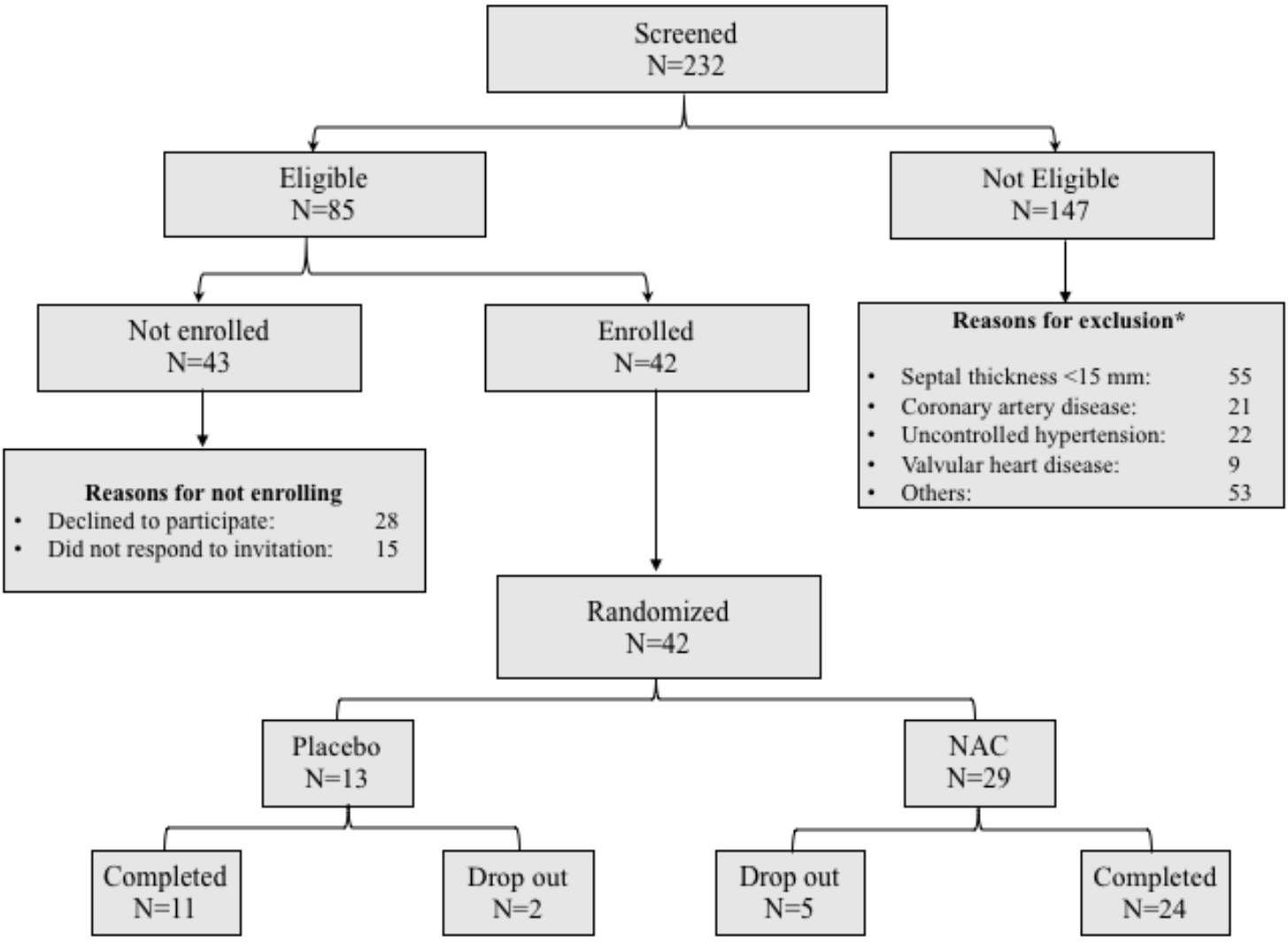
- Hypertrophic cardiomyopathy is caused mainly by mutations in genes encoding sarcomere proteins.
- Cardiac hypertrophy and fibrosis, important determinants of clinical outcomes, are considered secondary to the activation of numerous trophic and mitotic pathways, including oxidative stress-responsive mechanisms.
- Studies in animal models of HCM have shown that cardiac hypertrophy and fibrosis are reversible upon pharmacological interventions, including treatment with N-acetylcysteine (NAC), a precursor to glutathione.

What New Information Does This Article Contribute?

- "*H*ypertrophy Regression with N-*A*cety*L*cys*T*eine in *H*ypertrophic *C*ardio*M*yopathy" (HALT-HCM) is a double blind randomized, sex-matched, placebo-control study (N=42 patients) designed to test feasibility and determine effect sizes of NAC on indices of cardiac hypertrophy and fibrosis in patients with established HCM.
- Recruitment rate was slow but retention and compliance rates were high.
- About 2/3 of the participants had one or more pathogenic variants in genes known to cause HCM
- Treatment with a high dose of NAC for 12 months had negligible to modest effect sizes on indices of cardiac hypertrophy and fibrosis in patients with established HCM.

HCM is an important cause of sudden cardiac death in the young and heart failure with preserved ejection fraction. There is no effective pharmacological treatment for reversing or preventing cardiac hypertrophy and fibrosis in patients with HCM. Pharmacological interventions in animal models of HCM have shown reversibility of cardiac hypertrophy and fibrosis upon treatment with NAC. The findings in human patients, however, are notable for negligible or modest effect sizes on indices of cardiac hypertrophy and fibrosis. The small sample size of the study may have precluded firm conclusions about the efficacy of NAC in treatment of patients with HCM.

FIGURE 1



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*H*ypertrophy Regression with N-*A*cety*L*cys*T*eine in *H*ypertrophic *C*ardio*M*yopathy (HALT-HCM): A Randomized Placebo Controlled Double Blind Pilot Study

Ali J Marian, Yanli Tan, Lili Li, Jeffrey T Chang, Petros Syrris, Manouchehr Hessabi, Mohammad H Rahbar, James T Willerson, Benjamin Y Cheong, Chia-Ying Liu, Neal S Kleiman, David A Bluemke and Sherif F Nagueh

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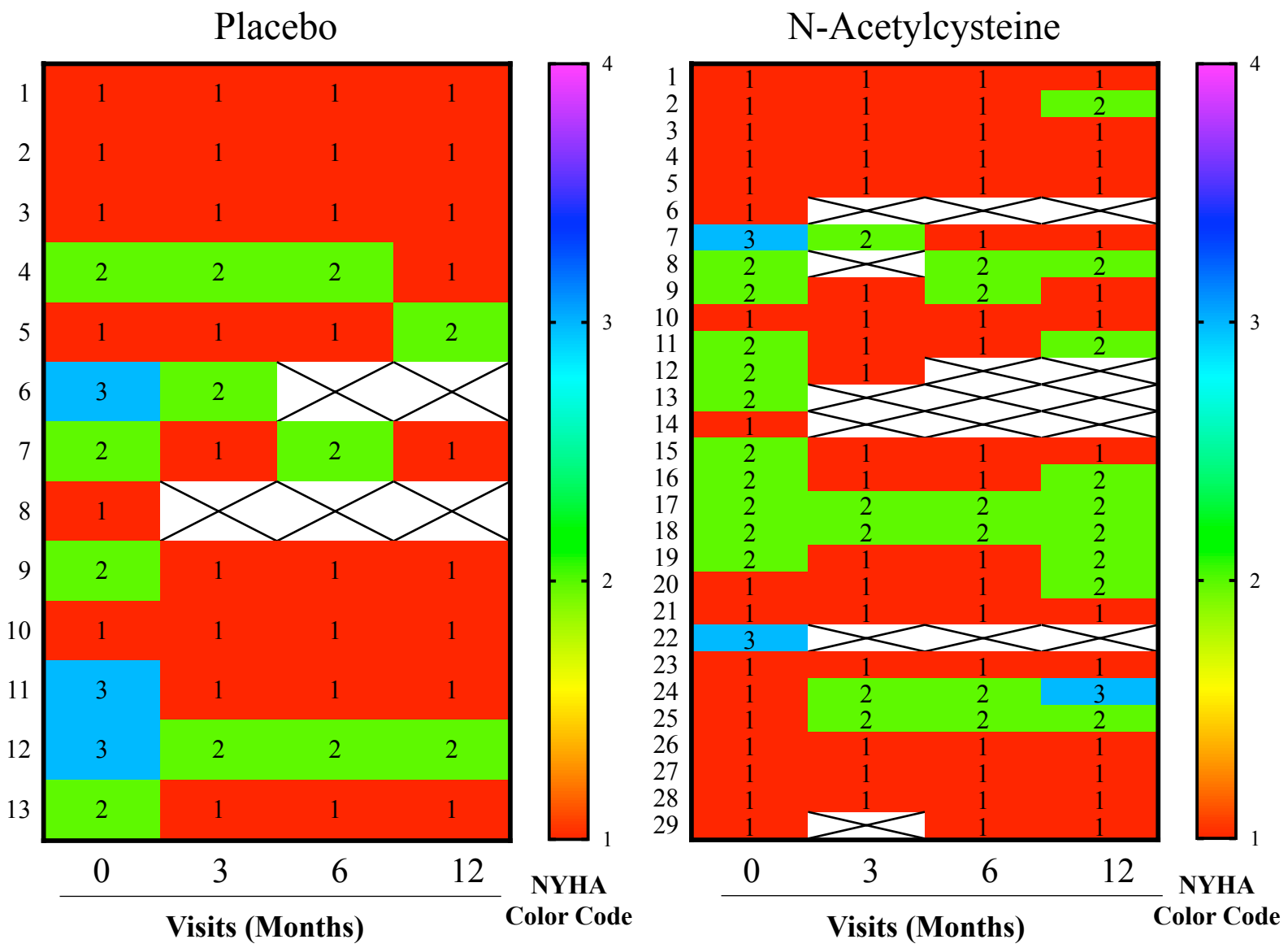
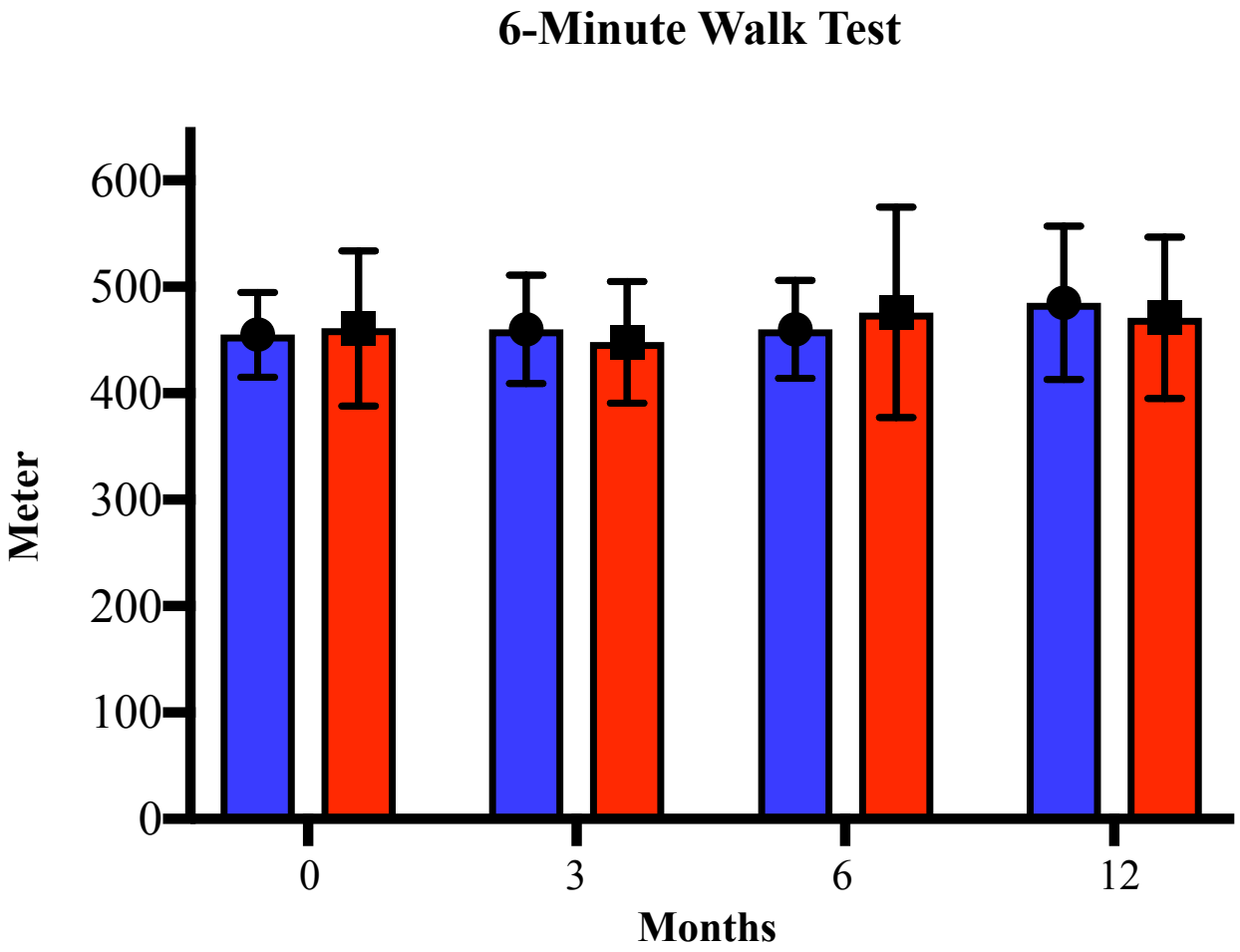


Online Figure I. Heat maps showing changes in New York Heart Association Functional Class in each individual at four time points. No significant difference was noted in any of the four time points in NYHA functional classes between the placebo and N-acetylcysteine groups. Each horizontal line indicate one individual. The number in each box indicates NYHA functional class. X indicates missing data.



Online Figure II. Distance (meter) walked in 6 minutes by the study participants. There were no differences between the two groups in the distance walked in 6 minutes at the baseline and three follow up time points. Mean and SD data are shown.
Blue: Placebo; **Red: N-acetylcysteine**

ONLINE TABLE I

New York Heart Association Functional Class at the Baseline and Follow up Time Points

	Placebo (N=13)				**NAC (N=29)**			
	Baseline	3 months	6 months	12 months	Baseline	3 months	6 months	12 months
Class I	5	9	8	9	16	18	18	14
Class II	5	3	3	2	11	5	6	9
Class III	3	0	0	0	2	0	0	1
Class IV	0	0	0	0	0	0	0	0

ONLINE TABLE II

Electrocardiogram Phenotypes (Intention-to-treat analysis)

	Placebo					NAC					Interaction
	Baseline		12 Months		*p*	Baseline		12 Months		*p*	*p*
N	13		11			29		24			
M/F	10/3		9/2			22/7		18/6			
Age (years)	47.6 (15.1)		50.35 (15.2)			50.7 (15.0)		51.15 (15.8)			0.04
Body weight (Kg)	89.3 (22.9)		89.4 (22.7)		0.22	92.6 (19.5)		92.9 (18.0)		0.14	0.75
BSA (m2)	2.07 (0.33)		2.07 (0.32)		0.16	2.12 (0.27)		2.12 (0.25)		0.31	0.93
Heart rate (bpm)	13	66.2 (11.8)	11	60.4 (11.4)	0.06	29	62.4 (11.2)	24	61.1 (10.7)	0.49	0.14
Atrial Fibrillation/ Flutter (N)	13	0	10	0	NA	28	1	22	1	NA	NA
Paced rhythm (N)	13	0	10	1	NA	28	1	22	3	0.15	0.94
Conduction defect (N)	13	4	10	2	0.24	28	9	22	9	0.32	0.12
Pathological Q waves (N)	13	1	10	1	0.84	28	5	22	2	0.43	0.54
Left ventricular hypertrophy (N)	13	6	10	7	0.27	28	20	22	19	0.16	0.71
QT interval (mean)	13	444.1 (37.7)	10	452.6 (33.0)	0.18	29	458.4 (37.4)	24	448.8 (37.0)	0.12	0.053
ST & T abnormalities (N)	11	12	10	8	0.44	28	20	22	16	0.61	0.47
LVH Score - Estes	13	6.2 (4.0)	10	6.2 (3.8)	0.23	29	5.7 (3.0)	24	6.0 (2.9)	0.70	0.48

All data are presented as mean (SD), unless specified

Abbreviations: M/F: Male/Female; NAC: N-acetylcysteine; BSA: Body surface area; LVH: Left ventricular hypertrophy;

ONLINE TABLE III

Echocardiographic Phenotypes (Intention-to-treat analysis)

	Placebo					NAC					Interaction
	Baseline		12 Months		*p*	Baseline		12 Months		*p*	*p*
N	13		11			29		24			
M/F	10/3		9/2			22/7		18/6			
Age (years)	47.6 (15.1)		50.35 (15.2)			50.7 (15.0)		51.15 (15.8)			0.04
Body weight (kg)	89.3 (22.9)		89.4 (22.7)		0.22	92.6 (19.5)		92.9 (18.0)		0.14	0.75
BSA (m2)	2.07 (0.33)		2.07 (0.32)		0.16	2.12 (0.27)		2.12 (0.25)		0.31	0.93
Echocardiographic Phenotypes	N	Mean	N	Mean		N	Mean	N	Mean		
IVST (mm)	13	17.08 (4.35)	11	17.82 (4.87)	0.94	29	18.45 (4.30)	24	17.92 (3.30)	0.34	0.44
LVPWT (mm)	13	13.08 (3.48)	11	13.27 (3.80)	0.92	29	12.41 (2.67)	24	13.79 (1.96)	0.008	0.15
Maximum wall thickness (mm)	13	19.77 (3.56)	11	20.54 (4.11)	0.98	29	20.00 (4.25)	24	19.75 (2.92)	0.39	0.39
LVEDD (mm)	13	40.15 (6.34)	11	39.91 (2.70)	0.93	29	40.03 (7.52)	24	39.83 (6.34)	0.98	0.99
LVESD (mm)	13	23.31(5.25)	11	21.55 (3.80)	0.35	29	22.24 (5.77)	24	21.88 (5.29)	0.79	0.57
LVEF (%)-mean	13	69.56 (6.03)	11	68.55 (5.17)	0.56	29	69.59 (6.22)	24	69.44 (6.12)	0.95	0.61
LA Volume (ml)	11	69.47 (27.02)	9	83.58 (27.83)	0.11	25	87.85 (28.00)	19	86.08 (31.63)	0.96	0.28
LVM (g)	13	281.66 (103.04)	11	290.44 (99.81)	0.98	29	270.78 (94.98)	24	281.98 (81.75)	0.41	0.67
LVMI (g/m2)	13	136.78 (49.65)	11	140.41 (47.27)	0.98	29	127.67 (42.70)	24	132.92 (35.41)	0.49	0.73
E (cm)	10	79.17 (16.25)	11	77.55 (14.24)	0.85	28	71.85 (19.00)	23	74.79 (22.40)	0.10	0.81
A (cm)	10	70.61 (23.40)	11	64.29 (31.31)	0.78	27	66.06 (26.20)	22	61.36 (24.09)	0.48	0.81
E/A ratio	10	1.27 (0.61)	11	1.40 (0.54)	0.61	27	1.22 (0.44)	22	1.38 (0.56)	0.16	0.88
IVRT (msec)	12	84.42 (12.96)	9	86.00 (18.77)	0.70	19	82.84 (12.90)	19	87.79 (20.86)	0.38	0.71
Septal Sa (cm/s)	10	5.42 (1.79)	7	4.69 (1.79)	0.03	18	5.19 (1.09)	16	4.26 (1.63)	0.046	0.67
Septal Ea (cm/s)	10	4.63 (1.98)	7	5.17 (1.48)	0.54	19	4.65 (1.44)	15	4.45 (1.61)	0.72	0.59
Lateral Sa (cm/s)	9	5.38 (2.18)	7	4.53 (1.69)	0.43	19	5.56 (1.64)	14	5.05 (1.84)	0.60	0.68
Lateral Ea (cm/s)	9	6.92 (2.51)	7	9.14 (3.27)	0.06	18	7.09 (2.25)	14	6.36 (2.35)	0.50	0.04

E/e' ratio	9	18.48 (8.57)	8	15.62 (6.33)	0.66	22	16.87 (8.62)	19	18.97 (9.59)	0.30	0.32
LVOT Gradient at rest (mmHg)	13	8.85 (21.76)	11	15.73 (28.44)	0.46	25	13.12 (23.48)	22	15.75 (25.02)	0.69	0.63
SAM: N (%)	10	1 (10.0)	10	4 (40)	0.23	20	6 (25)	19	5 (26.35)	0.85	NA

All data are presented as mean (SD), unless specified

Abbreviations: NAC: N-acetylcysteine; M/F: Male/females; CI: Confidence interval; SE: Standard Error; BSA: Body surface area; IVST: Interventricular septal thickness; LVPWT: Left ventricular posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVESD: Left ventricular end systolic diameter; LVEF: Left ventricular ejection fraction; LA: left atrium; LVM: Left ventricular mass; LVMI: Left ventricular mass indexed to body surface area; E: Mitral valve early inflow velocities; A: Mitral valve inflow late velocities; IVRT: Isovolumic relaxation time; Septal Sa: Systolic mitral annulus velocity measured at septal side of the mitral annulus; Septal Ea: Early diastolic mitral annulus velocity measured at septal side of the mitral annulus; Lateral Sa: Systolic mitral annulus velocity measured at lateral side of the mitral annulus; Lateral Ea: Early diastolic mitral annulus velocity measured at lateral side of the mitral annulus; LVOT: left ventricular outflow tract gradient; SAM: Systolic anterior motion of the mitral leaflet.

ONLINE TABLE IV

CMR Phenotypes (Intention-to-treat analysis)

	Placebo					NAC					Interaction
	Baseline		12 Months		*p*	Baseline		12 Months		*p*	*p*
N	9		8			15		10			
M/F	7/2		7/1			12/3		7/3			
Age (years)	47.6 (18.4)		51.1 (17.9)			48.0 (14.8)		49.9 (16.2)			0.22
Body weight (Kg)	84.2 (17.1)		85.8 (16.4)		0.45	90.4 (18.1)		93.6 (17.7)		0.08	0.27
BSA (m^2)	1.98 (0.24)		2.03 (0.24)		0.30	2.08 (0.25)		2.11 (0.25)		0.21	0.47
CMR	N	Means	N	Means		N	Means	N	Means		
MWT (mm)	9	16.40 (5.54)	8	17.58 (4.01)	0.38	15	16.14 (4.11)	10	17.75 (6.05)	0.37	0.80
LVEDV (ml)	9	175.48 (44.71)	8	175.13 (47.92)	0.21	15	177.20 (36.43)	10	179.00 (41.31)	0.60	0.51
LVESV (ml)	9	76.28 (26.65)	8	79.25 (30.89)	0.80	15	73.67 (17.01)	10	77.20 (24.29)	0.73	0.93
LVEF (%)	9	56.98 (8.55)	8	55.00 (10.95)	0.20	15	58.27 (6.19)	10	57.00 (8.03)	0.30	0.48
Mean LV mid wall strain (%)	8	-14.8 (3.50)	7	-15.86 (3.74)	0.16	15	-15.32 (2.24)	10	-14.78 (2.37)	0.32	0.04
Myocardial Mass (g)	7	191.86 (86.27)	7	221.29 (87.65)	0.85	14	211.21 (85.10)	9	238.22 (99.72)	0.33	0.56
EM (g)	7	48.00 (43.75)	7	51.43 (25.53)	0.75	14	27.57 (15.37)	9	38.00 (18.57)	0.14	0.35
EV (%)	7	21.29 (14.19)	7	22.29 (5.88)	0.89	14	12.93 (5.10)	9	16.67 (7.84)	0.30	0.59

All data are presented as Mean (SD), unless specified

Abbreviations: CMR; Cardiac magnetic resonance; NAC: N-acetylcysteine; M/F: Male/Female; BSA: Body surface area; MWT: Maximum wall thickness; LVEDV: Left ventricular end diastolic volume; LVESV: Left ventricular end systolic volume; LVEF: Left ventricular ejection fraction; LV: Left ventricle; EM: Enhanced myocardium; EV: Percent of myocardium that has scar (percentage of EM/MM)

Online Table V

Echocardiographic Phenotypes in Participants with Pathogenic Variants in *TTN* Gene

	Placebo			**NAC**			**p**	**$\Delta^{Placebo}$-Δ^{NAC}* (95% CI)**	**standard error**	**Effect size**
N	6			6						
M/F	5/1			4/2						
Age (year)	49.40 (13.17)			53.888 (16.50)						
	Baseline	**Follow-up**	**Delta (Δ)**	**Baseline**	**Follow-up**	**Delta (Δ)**				
Body weight (Kg)	88.98 (18.50)	91.41 (17.56)	2.42	90.65 (16.48)	91.78 (18.44)	1.14	0.62	1.29 (-6.90, 4.32)	2.52	0.32
BSA (m2)	2.08 (0.28)	2.12 (0.28)	0.03	2.1 (0.25)	2.08 (0.26)	0.02	0.66	0.02 (-0.10, 0.07)	0.04	0.32
IVST (mm)	18.17 (2.64)	17.50 (3.02)	-0.67	20.33 (3.98)	19.33 (3.78)	-1.00	0.77	0.33 (-2.75, 2.08)	1.09	0.24
LVPWT (mm)	11.50 (3.21)	12.50 (4.72)	1.00	12.50 (1.64)	14.33 (2.66)	1.83	0.65	0.83 (-3.17, 4.84)	1.80	0.22
Maximum wall thickness (mm)	19.33 (3.44)	19.17 (4.49)	-0.17	23.00 (4.82)	20.50 (2.74)	-2.50	0.28	2.33 (-6.87, 2.207)	2.04	0.70
LVEDD (mm)	37.50 (7.87)	39.83 (3.1)	2.33	41.00 (6.26)	42.33 (5.78)	1.33	0.77	1.00 (-8.44, 6.44)	3.34	0.15
LVESD (mm)	20.67 (5.47)	21.17 (2.4)	0.50	24.67 (6.02)	22.50 (7.37)	-2.17	0.32	2,67 (-8.31, 2.98)	2.53	0.70
EF (%)-mean	72.15 (4.56)	68.92 (5.46)	-3.23	68.68 (9.37)	68.72 (7.20)	0.03	0.44	3.27 (-5.82, 12.35)	4.08	0.58
LA Volume (ml)	82.82 (31.57)	87.67 (33.45)	9.48	92.38 (25.13)	90.70 (37.03)	0.14	0.72	9.34 (-57.22, 38.54)	20.77	0.46
LVM (g)	233.69 (95.11)	264.49 (118.99)	30.80	323.70 (108.44)	327.65 (106.04)	3.96	0.56	26.86 (-126.80, 73.10)	44.85	0.43
LVMI (g/m2)	109.18 (37.46)	123.17 (52.44)	13.98	157.15 (52.72)	156.08 (44.13)	-1.07	0.46	15.05 (-59.00, 28.90)	19.73	0.51
E (cm/s)	80.03 (20.20)	84.38 (12.87)	4.35	79.38 (18.69)	84.92 (21.36)	5.53	0.90	1.18 (-18.52, 20.90)	8.84	0.07
A (cm/s)	68.10 (25.55)	66.78 (31.41)	-1.32	69.56 (23.29)	63.46 (20.05)	-6.10	0.62	4.78 (-25.70, 16.14)	9.34	0.24
E/A	1.34 (0.72)	1.49 (0.68)	0.14	1.30 (0.37)	1.50 (0.49)	0.19	0.91	0.05 (-0.96, 1.06)	0.44	0.05
IVRT (msec)	79.33 (8.89)	83.75 (12.07)	2.25	72.75 (111.00)	98.40 (29.53)	16.00	0.16	13.75 (-29.98, 57.48)	17.01	0.74

Septal Sa (cm/s)	6.45 (2.02)	5.12 (1.99)	-1.05	5.30 (0.57)	3.66 (1.15)	-1.55	0.65	0.50 (-3.20, 2.20)	0.97	0.42
Septal Ea (cm/s)	5.50 (2.45)	5.36 (1.71)	-0.15	4.57 (1.00)	3.44 (0.88)	-1.00	0.32	0.85 (-3.19, 1.50)	0.84	1.10
Lateral Sa (cm/s)	5.46 (2.37)	4.68 (2.05)	0.28	5.04 (1.64)	6.20 (2.26)	-0.05	0.30	0.33 (-2.70, 2.05)	0.85	0.31
Lateral Ea (cm/s)	6.66 (1.60)	9.28 (3.98)	2.70	5.97 (2.04)	7.90 (4.10)	1.20	0.36	1.50 (-9.51, 6.51)	2.89	0.41
E/e' ratio	17.28 (10.25)	15.82 (7.47)	0.18	17.15 (3.48)	27.10 (7.80)	11.37	0.02	11.19 (1.21, 21.16)	4.08	2.41
LVOT Gradient at rest (mmHg)	8.50 (20.82)	15.73 (28.44)	0.33	3.75 (7.75)	17.60 (34.54)	24.33	0.15	24.00 (-62.03, 110.0)	13.10	29.39
SAM: N	1	1	0	0	0	0	NA	NA	NA	NA

All data are presented as mean (SD), unless specified

* Absolute value of $\Delta^{Placebo}$-Δ^{NAC}

Abbreviations: NAC: N-acetylcysteine; M/F: Male/females; CI: Confidence interval; SE: Standard Error; BSA: Body surface area; IVST: Interventricular septal thickness; LVPWT: Left ventricular posterior wall thickness; LVEDD: Left ventricular end diastolic diameter; LVESD: Left ventricular end systolic diameter; LVEF: Left ventricular ejection fraction; LA: left atrium; LVM: Left ventricular mass; LVMI: Left ventricular mass indexed to body surface area; E: Mitral valve early inflow velocities; A: Mitral valve inflow late velocities; IVRT: Isovolumic relaxation time; Septal Sa: Systolic mitral annulus velocity measured at septal side of the mitral annulus; Septal Ea: Early diastolic mitral annulus velocity measured at septal side of the mitral annulus; Lateral Sa: Systolic mitral annulus velocity measured at lateral side of the mitral annulus; Lateral Ea: Early diastolic mitral annulus velocity measured at lateral side of the mitral annulus; LVOT: left ventricular outflow tract gradient; SAM: Systolic anterior motion of the mitral leaflet.